

STUDIES ON THE CONSTITUENTS OF THE ROOTS OF *CELASTRUS PUNCTATUS*

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(Received 27th March 1997, revised Ms received 19th May 1997, accepted 22th May 1997)

Celastrus punctatus Thunb is a folk medicine in treatment of rheumatism in Taiwan. In the present study nine compounds have been isolated from the roots of *Celastrus punctatus*, namely β -amyrin, β -sitosterol, maytenfolic acid, β -sitosterol- β -D-glucoside, 3-hydroxyolean-12-en-22,29- γ -lactone, oleanolic acid, celastrol, kaempferol-3,7-O- α -L-dirhamnoside, and dulcitol. The structures of these compounds were identified either by MS and NMR determination with authentic samples or by comparison with the published data.

Key Words: *Celastrus punctatus*, triterpenoids.

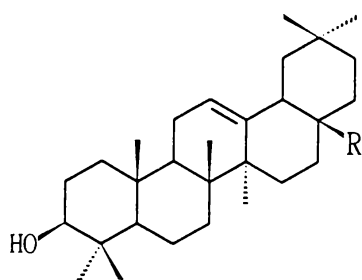
INTRODUCTION

The root of *Celastrus punctatus* Thunb of *Celastraceae* family has been used in treatment of rheumatism.^{1,2} It was reported to activate blood circulation and strengthen the muscle.³ In our previous screening tests, we found that the methanolic extract of the root showed strong analgesic and anti-inflammatory effects. The chemical constituents of this herb have never been reported in literature. For this reason, our recent studies on the constituents from *Celastrus punctatus* revealed the presence of several known compounds, we report now on the constituents of the roots of this plant.

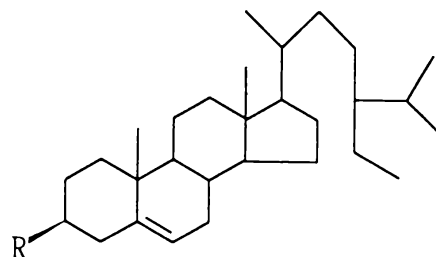
MATERIALS AND METHODS

Twenty-five kilos of *Celastrus punctatus* were collected from Nan-Tou County, Taiwan, and was identified by Mr. Nien Yuan Chiou, Senior Technician of China Medical College. The roots were extracted with methanol and concentrated, the methanolic extracts were partitioned successively with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol. After chromatographic separations by using silica gel and Sephadex LH-20, we obtained compound **1** and **2** from the *n*-hexane layer; compound **3**, **4**, **5** and **6** from the chloroform layer; compound **7** and **8** from the ethyl acetate layer; and compound **9** from the H₂O layer.

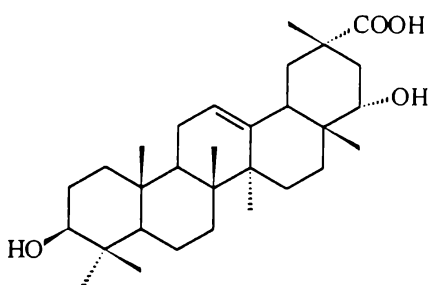
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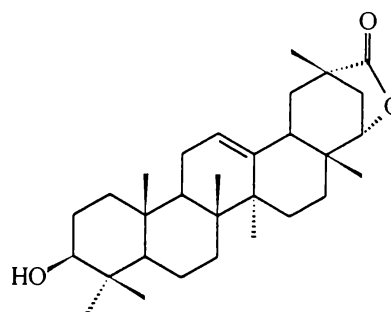
1 : R = CH₃
6 : R = COOH



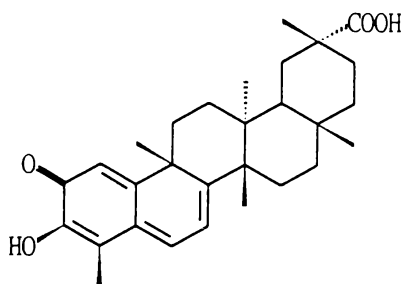
2 : R = OH
4 : R = O-glucose



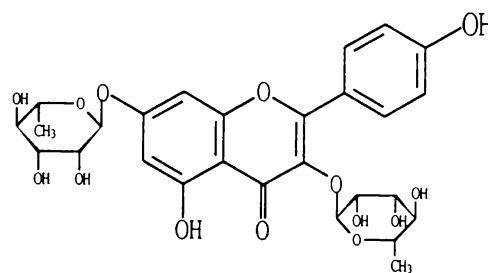
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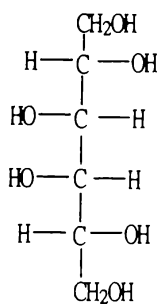
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7



8



9

The structure elucidation was performed by spectral analysis and/or comparison with the authentic samples.

β-Amyrin (1)

White needle crystal from *n*-hexane/ether, mp. 196-198 °C; EI-MS *m/z*: 426 (*M*⁺); ¹H-NMR (CDCl₃) δ ppm: 5.18 (t, 1H, *J* = 3.3 Hz, H-12), 3.22 (dd, 1H, *J* = 6.0 and 10.8 Hz, H-3), 1.13 (s, 3H, H-27), 0.99 (s, 3H, H-25), 0.96 (s, 3H, H-26), 0.93 (s, 3H, H-25), 0.87 (s, 6H, H-29, H-30), 0.83 (s, 3H, H-28), 0.79 (s, 3H, H-24); ¹³C-NMR (CDCl₃) δ ppm: 145.2 (C-13), 121.8 (C-12), 79.0 (C-3), 55.1 (C-5), 47.6 (C-9), 47.2 (C-18), 46.8 (C-19), 41.7 (C-14), 39.7 (C-8), 38.7 (C-4), 38.5 (C-1), 37.1 (C-22), 36.9 (C-10), 34.7 (C-21), 33.3 (C-29), 32.6 (C-7), 32.4 (C-17), 31.0 (C-

20), 28.3 (C-28), 28.0 (C-23), 27.2 (C-16), 26.9 (C-2), 26.1 (C-15), 25.9 (C-27), 23.6 (C-30), 23.4 (C-11), 18.3 (C-6), 16.7 (C-26), 15.5 (C-24), 15.4 (C-25).

β -Sitosterol (2)

White needle crystal from *n*-hexane/ether, mp. 140-142 °C; EI-MS *m/z*: 414 (M^+); $^1\text{H-NMR}$ (CDCl_3) δ ppm: 5.35 (br.d, 1H, $J = 5.4$ Hz, H-6), 3.53 (m, 1H, H-3), 1.25 (s, 1H, H-25), 1.01 (s, 3H, H-19), 0.92 (d, 3H, $J = 6.3$ Hz, H-21), 0.83 (d, 6H, $J = 6.6$ Hz, H-26 and H-27), 0.68 (s, 3H, H-18).

Maytenfolic acid (3)

White powder from $\text{CHCl}_3/\text{MeOH}$, mp. 309-311 °C; EI-MS *m/z*: 472 (M^+); $^1\text{H-NMR}$ ($\text{CDCl}_3/\text{CD}_3\text{OD}$) δ ppm: 5.25 (t, 1H, $J = 3.0$ Hz, H-12), 3.54 (dd, 1H, $J = 4.8$ and 12 Hz, H-16), 3.20 (t, 1H, $J = 7.5$ Hz, H-3), 1.26 (s, 3H, H-27), 1.17 (s, 3H, H-26), 0.994 (s, 3H, H-23), 0.991 (s, 3H, H-25), 0.98 (s, 3H, H-30), 0.95 (s, 3H, H-29), 0.19 (s, 3H, H-24); $^{13}\text{C NMR}$ ($\text{CDCl}_3/\text{CD}_3\text{OD}$) δ ppm: 180.57 (C-29), 142.78 (C-13), 123.03 (C-12), 78.46 (C-3), 74.75 (C-22), 54.91 (C-5), 47.28 (C-9), 46.14 (C-17), 42.21 (C-14), 41.72 (C-18), 39.59 (C-8), 38.40 (C-4), 38.35 (C-1), 36.55 (C-10), 32.20 (C-7), 27.59 (C-15), 26.41 (C-27), 24.07 (C-16), 23.22 (C-11), 18.72 (C-6), 16.32 (C-26), 15.17 (C-24), 15.05 (C-25).

β -Sitosterol- β -D-glucoside (4)

White powder from $\text{CHCl}_3/\text{MeOH}$, mp. 276-284 °C; FAB-MS *m/z*: 414 (M^+ -Sugar, aglycone), $^1\text{H-NMR}$ (DMSO-d_6) δ ppm: 5.33 (m, 1H), 4.88 (q, 2H, $J = 3$ Hz), 4.45 (t, 1H, $J = 4.8$ Hz), 4.21 (d, 1H, $J = 7.5$ Hz), 4.12 (dd, 1H, $J = 5.1$ Hz, 11.4 Hz), 3.16 (d, sugar-H, $J = 4.8$ Hz), 1.23 (s, 1H, H-25), 0.95 (s, 3H, H-19), 0.90 (d, 3H, $J = 6.3$ Hz, H-21), 0.81 (d, 3H, $J = 6.6$ Hz, H-26 and H-27), 0.65 (s, 3H, H-18).

3-Hydroxyolean-12-en-22,29- γ -lactone (5)

White powder from $\text{CHCl}_3/\text{MeOH}$, mp. 317-318 °C; FAB-MS *m/z*: 454 (M^+); $^1\text{H-NMR}$ (CDCl_3) δ ppm: 5.30 (t, 1H, $J = 3.6$ Hz, H-12), 4.16 (d, 1H, $J = 5.4$ Hz, H-22), 3.23 (m, 1H, H-30), 2.25 (s, 2H, H-27), 1.21 (s, 3H, H-27), 1.08 (s, 3H, H-26), 0.99 (s, 3H, H-23), 0.94 (s, 3H, H-25), 0.93 (s, 3H, H-30), 0.87 (s, 3H, H-28), 0.79 (s, 3H, H-24); $^{13}\text{C-NMR}$ (CDCl_3) δ ppm: 182.54 (C-29), 140.25 (C-13), 124.67 (C-12), 83.13 (C-22), 78.89 (C-3), 55.17 (C-5), 48.91 (C-9), 47.48 (C-19), 43.38 (C-18), 42.48 (C-14), 39.57 (C-8), 39.48 (C-4), 38.71 (C-1), 37.02 (C-10), 33.11 (C-7), 28.09 (C-23), 27.02 (C-27), 24.88 (C-28), 23.99 (C-11), 22.26 (C-21), 18.25 (C-6), 16.91 (C-26), 15.57 (C-24), 15.36 (C-25).

Oleanolic acid (6)

White needle crystal from *n*-hexane/ether, mp. 308-310 °C; EI-MS *m/z*: 456 (M^+); $^1\text{H-NMR}$ (CDCl_3) δ ppm: 5.26 (t, 1H, $J = 3.3$ Hz, H-12), 3.18-3.23 (m, 1H, H-3), 2.80 (dd, 1H, $J = 3.8$ Hz and 13.3 Hz, H-18), 1.23 (s, 3H, H-27), 1.10 (s, 3H, H-25), 0.96 (s, 3H, H-26), 0.87 (s, 6H, H-29, H-30), 0.75 (s, 3H, H-28), 0.72 (s, 3H, H-24); $^{13}\text{C-NMR}$ (CDCl_3) δ ppm: 183.75 (C=O), 144.07 (C-13), 123.12 (C-12), 79.51 (C-3), 55.68 (C-5), 48.09 (C-9), 46.99 (C-17), 46.34 (C-19), 42.06

(C-14), 41.44 (C-18), 39.74 (C-8), 39.23 (C-4), 38.86 (C-1), 37.56 (C-10), 34.26 (C-21), 33.54 (C-29), 33.07 (C-7), 32.90 (C-22), 31.15 (C-20), 28.57 (C-23), 28.15 (C-15), 27.64 (C-2), 26.41 (C-27), 24.05 (C-30), 23.87 (C-16), 23.39 (C-11), 18.76 (C-6), 17.61 (C-26), 16.02 (C-24), 15.79 (C-25).

Celastrol (7)

Red needle crystal from *n*-hexane/benzene, mp. 206-207 °C; EI-MS *m/z*: 450 (*M*⁺); ¹H-NMR (CDCl₃) δ ppm: 7.06 (d, 1H, *J* = 7.5 Hz, H-6), 6.49 (s, 1H, H-1), 6.33 (d, 1H, *J* = 7.5 Hz, H-7), 2.21 (s, 3H, H-23), 1.43 (s, 3H, H-24), 1.27 (s, 3H, H-29), 1.24 (s, 3H, H-29), 1.09 (s, 3H, H-27), 0.57 (s, 3H, H-26); ¹³C-NMR (CDCl₃) δ ppm: 182.39 (C-28), 178.39 (C-2), 172.70 (C-8), 165.12 (C-10), 147.01 (C-3), 135.45 (C-6), 127.54 (C-5), 120.54 (C-1), 120.33 (C-7), 118.31 (C-4), 45.27 (C-14), 44.22 (C-18), 43.03 (C-9), 39.89 (C-20), 39.26 (C-13), 38.29 (C-24), 36.29 (C-16), 34.43 (C-22), 33.69 (C-11), 32.35 (C-29), 31.41 (C-27), 30.99 (C-19), 30.60 (C-17), 29.4 (C-21), 29.25 (C-12), 28.66 (C-15), 21.39 (C-25), 18.64 (C-26), 10.37 (C-23).

Kaempferol-3,7-O-α-L-dirhamnoside (8)

Yellow powder from MeOH/CHCl₃, mp. 220-223 °C; EI-MS *m/z*: 579 (*M*+*H*)⁺; ¹H-NMR (CD₃OD) δ ppm: 7.77 (d, 2H, *J* = 9 Hz, H-2',6'), 6.92 (d, 2H, *J* = 8.7 Hz, H-3',5'), 6.69 (d, 1H, *J* = 2.1 Hz, H-8), 6.44 (d, 1H, *J* = 2.1 Hz, H-6), 5.56 (s, 1H), 5.38 (d, 1H, *J* = 1.8 Hz), 4.63 (s, 1H), 4.24 (m, 1H), 4.03 (m, 1H), 3.85 (d, 1H, *J* = 3.3 Hz), 3.82 (d, 1H, *J* = 3.6 Hz), 3.59 (d, 1H, *J* = 6.6 Hz), 1.26 (d, 3H, *J* = 6 Hz), 0.93 (d, 3H, *J* = 5.7 Hz); ¹³C-NMR (CD₃OD) δ ppm: 179.8 (C-4), 163.6 (C-7), 163.1 (C-5), 161.8 (C-4'), 159.8 (C-9), 158.1 (C-2), 136.5 (C-3), 132.1 (C-2',6'), 122.4 (C-1'), 116.6 (C-3',5'), 107.6 (C-10), 103.5 (Rha), 100.6 (Rha), 99.8 (C-6), 95.6 (C-8), 73.6 (Rha), 73.2 (Rha), 72.1 (Rha), 71.9 (Rha), 71.6 (Rha), 71.3 (Rha), 18.1 (Rha), 17.6 (Rha).

Dulcitol (9)

White powder from MeOH/H₂O, mp. 188-189 °C; EI-MS *m/z*: 182 (*M*⁺); ¹H-NMR (D₂O) δ ppm: 4.07 (t, 2H, *J* = 6.3 Hz, H-3 and H-4), 3.78 (d, 6H, *J* = 7.5 Hz, H-1, H-6, H-2, and H-5); ¹³C-NMR (D₂O) δ ppm: 70.36 (C-3, C-4), 69.56 (C-2, C-5), 63.42 (C-1, C-6).

RESULTS AND DISCUSSION

We have obtained nine compounds from the root of the *Celastrus punctatus* Thunb, all the nine compounds were first isolated from *Celastrus punctatus*. Compound **1** and **2** were isolated from the *n*-hexane layer. Compound **3**, **4**, **5** and **6** were isolated from the chloroform layer. Compound **7** and **8** were isolated from the EtOAc layer, and compound **9** was obtained from the water layer.

Compound **1** is a triterpene, and identified as β-amyrin in comparison of the MS and NMR spectra with the reported literature.⁴ Compound **2** is identified as β-sitosterol by comparison with the published data⁵ which is a typical phytosterol usually existing in the common plants.

Compound **3** shows the molecular peak at 472 in the MS spectrum and possesses a carboxylic acid and two hydroxyl-group which were supported by NMR experiments. An olean-12-ene system from the signals at δ 123.03 (C-12) and δ 142.78 (C-13)⁶ was also obtained, and the

compound was confirmed as maytenfolic acid by comparing of the MS and NMR spectra with the reported literature.⁶

The ¹H NMR spectrum of compound **4** is similar to compound **2** and also shows a proton signal of glucose at δ 4.88 (d, $J = 8$ Hz), thus the β -sitosterol is connected with D-glucose at β position in according to the J value.⁵ Compound **4** was identified to be β -sitosterol- β -D-glucoside.

There are many similarities between compound **3** and **5** of their NMR spectrum. In comparison between compound **3** and **5**, signal of C-29 shows a chemical shift from δ 180.57 (**3**) to δ 182.54 (**5**). This means that in compound **5**, the carboxylic acid group and hydroxyl group were cyclized and becomes a triterpenoidal γ -lactone ring naturally between C-20 and C-29.⁶ Compound **5** was identified as 3-hydroxyolean-12-en-22,29- γ -lactone by comparing with published data.⁶

Compound **6** is white needle crystal with molecular peak at m/z 456, and also a triterpene. It was identified as oleanolic acid in comparison of the MS and NMR spectra with the reported literature.⁷

Compound **7** is a red needle crystal with molecular peak at m/z 450. The existence of the fragments at m/z 201 and m/z 238 in the mass spectrum suggested it to be celastrol.⁸ NMR experiments also supported to the same conclusion.

Compound **8** is a flavonoid glycoside. The ¹³C-NMR signals at δ 17.6 and δ 18.1 in the glycones indicated the two methyl groups of rhamnose,⁹ and all other NMR signals are similar to the values with the literature reported.⁹ Compound **8** was identified to be kaempferol-3,7-O- α -L-dirhamnoside.

Compound **9** is identified as dulcitol by comparison of the MS and NMR spectra with authentic sample, and a hexose alcohol existing widely among the *Celastraceae* family.²

ACKNOWLEDGEMENT

We are very grateful to Mr. Nien-Yuan Chiou, China Medical College for identification of this plant and to National Science Council of R.O.C. for grant support to Yuan Shiun Chang (NSC-82-0420-B-039-017-M13).

REFERENCES

1. Kan WS. Manual of Medicinal Plants in Taiwan Vol 1-3, National Research Institute of Chinese Medicine, Taipei, pp 470-471, 1985.
2. Kan WS. Pharmaceutical Botany, National Research Institute of Chinese Medicine, Taipei, pp 369-371, 1969.
3. Chiu NY. Alpine Medicinal Plants of Taiwan, SMC Publishing Inc, Taipei, pp 89, 1993.
4. Ted KC, Diane CA, Norman CB, David FW. Anti-repellent Triterpenoids from *Cordia alliodora*, J Org Chem 48(20):3225-3231, 1983.
5. Lin WN. The Chemical Constituents of *Rhododendron ellipticum* MAXIMOWICZ, Graduate Institute of Chinese Pharmaceutical Sciences, China Medical College, Taichung, pp 24-25, 1991.
6. Nozaki H, Suzuki H, Lee KH, McPhail AT. Structure and Stereochemistry of Maytenfolic

- Acid and Maytenfoliol, Two New Antileukemic Triterpenes from *Maytenus diversifolia*: X-Ray Crystal Structure, J Chem Soc Chem Commun 1048-1051, 1982.
7. Pouchert CJ, Behnke J. The Aldrich Library of ^{13}C and ^1H FT NMR Spectra III. Aldrich Chemical Company 1993; 595 B.
 8. Gamlath CB, Gunatilaka AAL, Tezuka Y, Kikuchi T. The Structure of Celastranhydride: A Novel Triterpene Anhydride of Celastraceae, Tetrahedron Letters Vol. 29:109-112, 1988.
 9. Agrawal PK. Carbon-13 NMR of Flavonoids, Elsevier Science Publishers B.V., Amsterdam, 1989.

光果南蛇藤 (Celastrus punctatus Thunb) 之成分分離

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(1997 年 3 月 27 日受理, 1997 年 5 月 19 日收校訂稿, 1997 年 5 月 22 日接受刊載)

光果南蛇藤是台灣著名民間藥,「大鐵牛入石」的來源植物之一,民間一般襲用其治療風濕關節腫痛、腰痛、經閉等。在初步的藥理試驗中發現,光果南蛇藤之甲醇抽出物對於福馬林所誘導之小鼠後蹠疼痛具有良好之抑制作用,此外亦有對抗腫瘤及抗凝血作用。南蛇藤屬植物於以往的文獻中已有十三種植物提出成分分析報告,此外亦顯示此屬植物具有良好的抗菌及抗病毒作用,而光果南蛇藤至今仍未有成分分離之文獻報告,故而本實驗就此植物進行成分之分析。

光果南蛇藤根部經由抽取、分離、純化等工作後,發現了九種化學成分,分別為 β -amyrin、 β -sitosterol、maytenfolic acid、 β -sitosterol- β -D-glucoside、3-hydroxyolean-12-en-22,29- γ -lactone、oleanolic acid、celastrol、kaempferol-3,7-O- α -L-dirhamnoside 及 dulcitol,這些化合物之結構式係經光譜分析或樣品比對或文獻資料而加以確認,且成分之藥理效用已進一步評估。

關鍵詞：光果南蛇藤,南蛇藤屬。